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High-pressure crystal structure analysis of 3-isopropylmalate dehydrogenase from piezosensitive and piezophilic *Shewanella* species

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Introduction

Organisms living in deep seas such as in the Mariana Trench must be adapted to extremely high-pressure environments. For example, a protein 3-isopropylmalate dehydrogenase (IPMDH) from the deep-sea bacteria *Shewanella benthica* DB21MT2 (SbIPMDH) remains nearly 80% active under 150 MPa conditions, whereas IPMDH from the land bacteria *S. oneidensis* MR-1 (SoIPMDH) becomes inactivated [1]. In order to unravel the differences existing between these two IPMDHs, we attempted to solve their structures while applying a high-pressure protein crystallography (HPPX) method by means of a diamond-anvil cell (DAC).

High-pressure induced effects such as dissociation of oligomeric proteins, structural transition or denaturation are examples of the properties particular for these proteins. Previously published reports assumed that they are induced by water penetration into the hydrophobic interior of proteins. Although concrete structural data are still lacking, several theoretical and simulation studies support this theory [2]. To obtain structural information of these key steps, we assume that the HPPX method will play a pivotal role.

Material and Methods

SoIPMDH and SbIPMDH were overexpressed in *E. coli* using pQE80L vectors. The crystals of SoIPMDH- or SbIPMDH-IPM complexes were obtained by hanging-drop vapor diffusion method by mixing protein solution consisting of 15 mg mL^{-1}, 10 m*M* magnesium chloride, 10 m*M* IPM, and 10 m*M* Tris-HCl (pH8.0) with reservoir solutions consisting of 11%(*w/v*) PEG 3350, 200 m*M* calcium acetate, and 100 m*M* HEPES sodium (pH 7.0), or 15%(*w/v*) PEG 3350, and 100 m*M* HEPES sodium (pH 6.7), respectively. The crystals were mounted in a DAC and slowly compressed. The solutions used as pressure media consisted of 18%(*w/v*) PEG 3350 in 100 m*M* HEPES sodium (pH 7.0), and 200 m*M* calcium acetate, or 32%(*w/v*) PEG 3350 in 100 m*M* HEPES sodium (pH 6.7), respectively. The actual pressure in the pressure chamber was determined using the fluorescence emanating from a ruby ball loaded into the chamber together with the sample. To make HPPX measurements possible at the beamline PF AR-NW12A, several equipments were modified to accept the DAC sample holder, such as the goniometer head and the direct beam stopper. Using such settings, the crystal structures of SoIPMDH- and SbIPMDH-IPM complexes have been determined at about 2 Å resolution under several pressures up to several hundreds MPa. Data were collected using a wavelength of λ = 0.7 Å, an exposure time of 5 - 10 seconds per image, and with an oscillation angle of 1 degree. Integrated intensities produced by *DENZO* were scaled and merged using *SCALA* implemented in the *HKL2000* suite. The structures were determined by the molecular replacement method using *Molrep* and further refined with *Refmac5*.

Results and Discussion

Pressure dependences of the molecular volume of SoIPMDH and SbIPMDH dimer are uniform. The compressibility of the molecular volume is 4.4 x 10^{-2} and 8.4 x 10^{-2} GPa^{-1}, respectively. Unexpectedly, the compressibility of SbIPMDH-IPM is larger than those of SoIPMDH-IPM.

In order to reveal the mechanism of pressure tolerance of SbIPMDH, we will focus on some differences in the activation volume of SoIPMDH and SbIPMDH. Hence, it will be necessary to determine the structures of both enzymes in their apo as well as holo states. Work in this direction is in progress.

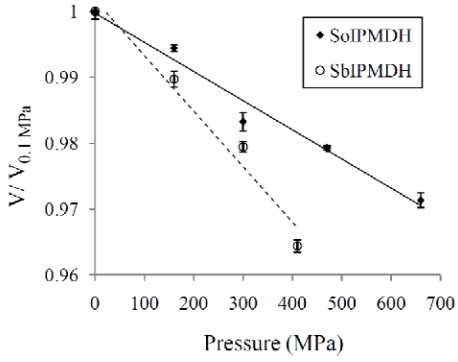

Figure.
Relative volumes of the SbIPMDH and SoIPMDH dimer

References

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